ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Osteoporosis and the Role of Soybeans in Ovariectomized (OVX) Female Albino Rats

Serag El Din, O. S.; Batta, H. Abd El Azim ; Rania, A. Lotfy

Zoology Department, Faculty of Women for Art, Science & Education Ain Shams University

Manuscript Info

Manuscript History:

Received: 14 April 2015
Final Accepted: 22 May 2015
Published Online: June 2015

Key words:

Soybeans , Osteoporosis, Ovariectomized, Female,Thyroid gland ,bone

Corresponding Author

Batta, H. Abd El Azim

Abstract

Menopause drastically increases the risk of osteoporosis. Aside from drug therapy, lifestyle and nutritional factors play an important role in the maintenance of skeletal health. The objective of this study was to investigate the effects of soybean on bone health, and to discuss the role of soybean isoflavones on osteoporosis in ovariectomized (OVX) rats. A total of 105 female Wister albino rats having an average weight of 140-150 gm were used. They were divided into three control and three treated groups. The three control groups were: normal control, sham operated control (positive control) and OVX control (negative control), each group consisted of 20 animals. The treated groups, 15 animals each, received soybean (30, 60, 90 gm/70kg/human b.w) which corresponds to the three doses (60, 120, 190 mg/145 gm rat b.w) daily, then were left on AIN-93M diet and fresh water adlibitum for three months Compared to OVX-control group, OVX female rats fed on soybean showed significant increase in femur bone weight, femur bone Ca++ and serum Ca++. This was accompanied with a decrease in serum PTH. There were little differences in serum total E2 in the three treated groups. Free T3 and free T4 decreased but Serum TSH significantly increased in in OVX female rats fed only on 190 gm soybean/145 gm rat b.w . These results may indicate that soybean may have adverse effects on bone health and thyroid gland function resulting in the suppression of thyroid hormone synthesis in ovariectomized female albino rats.

INTRODUCTION

future trials that combine these phytonutrients with established bone nutrients (i.e. calcium and vitamin D) are needed to determine whether combined strategies can produce more robust effects on skeletal health(**Sandra etal. ,2013**).

The fermented soybean-based foods have played an important role in traditional diets around the world for many centuries, and *Bacillus subtilis* is typically used in the fermentation of soybean-based foods **(Lin et al.,2012).**

The word "menopause" literally means the "end of monthly cycles" from the Greek words: pausis means "cessation" and the word men from mensis meaning "monthly".

Menopause is a period normally occupying one-third of women's life. Reduced bone density is one of the most prominent symptoms during menopause **(Karnataka, 2008)**.

Osteoporosis is one of the most important endocrine diseases, characterized by bone resorption which exceeds its formation, an imbalance between the activity of osteoclast and osteoblasts, reduce bone mass and structural deterioration of bone tissue with a resulting increase in bone fragility and fracture risk **(Nussey and whitehead, 2001; Chan *et al*., 2003; Barlow, 2007; Hohenhaus *et al*., 2007** and **Levine *et al*., 2007).** Hormones, such as osteoblast to maintain mineral homeostasis and to influence a variety of bone cell functions **(Marie, 2001** and **Strewler, 2001)**. Increased PTH secretion leads to an increase in bone resorption and contributes to general skeletal demineralization, which is related to estrogen deficiency and requires suitable medical treatment (**Silverberg and Bilezikian, 1994** and **McKane *et al*., 1997).** Currently available treatments for postmenopausal osteoporosis include hormone replacement therapy (HRT). HRT has traditionally been used for treatment of menopausal disorders **(WHI, 2002** and **Ahlborg *et al*., 2004)**. Estrogen play a key role in the maintenance of the adult skeleton (**Heaney, 2002; Riggs *et al*., 2002** and **Nelson et al., 2005**). It acts as a potent anabolic steroid concerning bone mass (via estrogen receptor, ERα and ERβ on bone cells) by directly suppressing bone resorption (**Setchell and Lydeking-Olsen, 2003**). Indirectly, estrogen is also thought to exert skeletal effects through enhancement of intestinal calcium absorption by way of its trophic effect on $1,25(OH)_2$ vitamin D_3 and perhaps independently (**Perez *et al*., 2008**). However, not all women can, or prefer to take HRT. Side-effects such as breast, endometrial and ovarian cancer, cardiovascular disease, cholethiasis, breast tenderness, mood changes, venous thromboembolism, pulmonary embolism and ischemic stroke may result **(Katzung, 2001; Nussey and Whitehead, 2001; Beck *et al*., 2003; Hays *et al*., 2003** and **Peverill, 2003)**. As a result of the adverse effects of HRT, many authors have shown the interest in phytoestrogen, a group of plant-derived compounds which are structurally and functionally similar to estradiol. Many foods contain phytoestrogens, but soybeans are particularly rich in isoflavones, one of the common classes of phytoestrogens.

Soybeans (*Glycine max*) are unique foods because of their rich nutrient content. These legumes are composed of macronutrients such as lipids, carbohydrates and proteins. Soybean also contains micronutrients, which include isoflavones, phytate, saponins, phytosterol, vitamins and minerals.

Isoflavones are mainly found in soybean, the most important dietary source of phytoestrogens for humans, cattles and rodents. The main isoflavones found in soybeans are genistein, daidzein, glycitein and their respective acetyl, malonyl and aglycone forms **(Lee *et al*., 2003** and **Kim *et al*., 2005)**. Isoflavones may have antiatherosclerotic, antioxidative, anti-tumorial and antiestrogenic activities **(Scheiber *et al*., 2001; Messina, 2002** and **McCue and Kalidas, 2004)**. The structural similarity of isoflavones to estrogens enable them to bind to estrogen receptors and provide them the ability to mildly mimic and in some cases act as antagonist to estrogen on tissues (e.g. breast and endometrium) **(Nussey and Whitehead, 2001; Hurst, 2002** and **Cassidy and Dalais, 2003)**. When the level of estradiol is low, as it is in menopause, isoflavones exert more agonist properties (e.g. on bone and plasma lipid) and thus mildly mimic endogenous estrogen actions **(Collins *et al*., 1997** and **Setchell and Cassidy, 1999)**.

Role of soybean on osteoporosis Alekel *et al*. (2000);Clifton-Bligh *et al*. (2001); Setchell and Lydeking-Olsen (2003); Crisafulli *et al*. (2004); Cassidy *et al*. (2006);Ye *et al*. (2006); Marini *et al*. (2007); Ma *et al*. (2008); Marini *et al*. (2008); Atmaca *et al*. (2008) and **Taku *et al*. (2010)** said that consumption of 90 – 126 mg isoflavones/day for short duration (less than three months) inhibits bone resorption and stimulates bone formation so that isoflavones have beneficial effects on bone mineral density and bone mechanical strength in postmenopausal women.

Shi and Su (2000) suggested that soy proteins with isoflavones attenuated bone loss, increased calcium retention and decreased calcium excretion in OVX rats.

On the other hand dietary supplementation of isoflavones have minimal or did not have synergistic effects on bone resorption, bone turnover, bone mineral density and calcium metabolism in postmenopausal women **(Wangen *et al*., 2000; Cheong *et al*., 2006; Brink *et al*., 2008 , Kenny *et al*., 2009 and Azza etal.,**

2014), in OVX rats (**Picherit *et al*.,2001; Breitman *et al*., 2003; Picherit *et al*., 2003; Fonseca and ward, 2004; B**

Epidemiological studies suggest that consumption of soybeans or soy foods has been related to decreasing the risk of getting some types of breast and prostate cancer, cardiovascular diseases or osteoporosis **(Park et al.,2012).**

ahr *et al*., 2005; Nakai *et al*.,2005 and **Figard *et al*.,2006)** and in OVX monkeys **(Jayo *et al*., 1996).**

Zhang *et al*. (2001) said excessive soy intake has been reported to be responsible for the development of goiter, including thyroid enlargement in rodents. this findings suggest that intake of soy may reduce the efficiency of thyroid hormone function and that soybeans may contain goitrogens that can interfere with the utilization of iodine or functioning of the thyroid gland and cause thyroid problems.

Jayagopal *et al*. (2002) consuming soy protein isolate (SPI) of varying isoflavone content suggested that it decreased free T4.

Panda *et al*. (2009) reported that soy sterols, at a moderate concentration (5 mg/kg) for 21 days significantly increaseed serum T4 level but reduced the levels of serum T3 in female mice

On the other hand Bitto et al. (2010) said that daily consumption of genistein for three years had no effect on thyroid hormones, did not alter the function of enzymes involved in thyroid hormone production, did not cause any changes in thyroid hormone auto-antibodies, and had no effect on the expression of thyroid hormone receptors in postmenopausal women.

Therefore, the present study aims to investigate the role of soybean on different biochemical parameters, in OVX female albino rats.

2-Material and methods

2.1- Experimental animals:-

A total of 105 female Wister albino rats, weighing between 140-150 gm were used in the present study. The animals were obtained from the private market Abou-Rawash, Giza, Egypt. They were housed in an environmentally controlled laboratory and acclimatized for one week before the onset of the experiment; they were kept in cages and provided with vegetables and tap water ad libitum.

Following this week of adaptation, rats were divided into three control groups and three OVX treated groups. The three control groups were: normal control (n=20), sham operated control without ovariectomy which is positive control (n=20) and OVX control which is negative control (n=20). The treated group, 15 animals each, received cooked soybean with three different doses (30, 60, 90 gm/70kg b.w.) (i.e. 60, 120, 190 mg/145 gm rat b.w.) daily for three months then were left on AIN-93M diet (according to **Reeves *et al*., 1993**) and fresh water ad libitum.

The dose rates in the experiments were computed according to the average weight of the rats and were proportional to the human dose rate.

2.2- Surgical procedures:-

Under general anesthesia, bilateral ovariectomy was performed by ligation and excision of both ovaries. The sham-operated rats were treated in a similar way, but only the ovaries and oviducts were manipulated.

Estrogen content was examined every week after ovariectomy to ensure that it declined to its level in menopause.

2.3- Soybean diets:-

Soybean (type: Giza 22) were purchased from Agriculture Research Center, Giza, Egypt. Soybean seeds were soaked for 12 hours at room temperature then were cooked at 100°C for 30 minutes to decrease the amount of the anti-nutrients.

2.4 - Sample collection:-

2.4.1- Body weight and absolute organs weight analyses:-

Body weight of rats were recorded before and after the experimental period. After sacrifice left femur bone was obtained , weighed and placed in 1:1 ether & alcohol .

2.4.2-Blood samples.

At the end of each experiment blood was withdrawn from the orbital plexus of the eye or cardiac puncture and serum was separated to be used for the estimation of the following parameters:

1. Determination of serum total calcium by the colorimetric method according to **(Gindler, 1972)** using Bio-diagnostic Co kits.

2. Determination of calcium content in bone according to **(Puntheeranurak *et al*., 2006)** and **Charoenphandhu *et al*., 2007).**

3. Determination of serum total estradiol using radioimmunoassay kits (Siemens Medical Solutions Diagnostics, USA) according to **(Xing *et al*., 1983).**

4. Determination of serum PTH using enzyme immunoassay kits (Siemens Medical Solutions Diagnostics, USA) according to **(Kao *et al.*, 1992)).**
5. Determination of serum free T3 using enzyme immunoassay kits **(**Siemens Medical Solutions Diagnostics, USA**)** according to **(Young et al, 1975).**
6. Determination of serum free T4 using enzyme immunoassay kits (Siemens Medical Solutions Diagnostics, USA) according to **(Liewendhal, 1990).**
7. Determination of serum TSH using radioimmunoassay kits (Siemens Medical Solutions Diagnostics, USA) according to **(Baloch et al., 2003).**

2.4.3- Statistical analysis:

Statistical analysis was evaluated by using student "T" test. Analysis of variance (ANOVA) was also used to assess the significance of differences among various treated groups. Statistical processor system support "SPSS" for windows soft ware, release 10.0 (SPSS, Chicago, IL) was used.

3.Results

3.1 Analysis studies:

Table (I) show the effect of different doses of soybean on femur bone weight, femur bone Ca++, serum Ca++ , E2 and PTH of ovariectomized female albino rats treated for three months.

The present data indicate that in comparison to the sham-operated group, OVX control groups showed significant decrease of femur bone weight , femur bone Ca++ , serum Ca++ and total estradiol , but, increase in PTH

Compared to OVX-control group, OVX female rats fed on 60 , 120 and 190 mg soybean/145 gm/ rat b.w. showed significant increase of femur bone weight, femur bone Ca++ , serum Ca++ , femur bone weight , femur bone Ca++ and and for serum Ca++ . This was accompanied with a decrease in serum PTH . Total E2 in OVX female rats fed on 60 ,120 and 190 mg soybean/145 gm /rat b.w. showed slight increase .

Table(II)show the effect of different doses of soybean on serum TSH and thyroid hormones of ovariectomized(FT_3 ,FT_4) female albino rats treated for three months.

The present data indicate that in comparison to the sham-operated group, OVX showed slight decrease of FT_3 and FT_4 as well as a slight increase of serum TSH .

Compared to OVX-control group ,with OVX female rats fed only on 190 gm soybean/145gm/rat b.w. showed significant decrease of serum FT_3 and T_4 . This was accompanied by a significant increase of serum levels of TSH in OVX female rats of the same group

3.2 Histopathological studies:

Fig. (1) Shows Histopathological studies of thyroid gland, normal control of rat of with normal histological structure of the active follicles (F) with cubodial lining epithelium (arrow). **(H&E x 250**)

Fig. (2) Photomicrograph of thyroid gland of OVX-control rat showing inactive cystic dilatation with massive amount of colloid in the follicular lumen (C) and flattened lining epithelium (arrow). **(H&E x 250**)

Fig. (3) Photomicrograph of thyroid gland of treated OVX rat with 60 mg soybean / rat b.w. showing cubodial lining epithelium of the follicles (arrow), atrophy and obliteration in some follicles (A) while other containing eosinophilic colloid (C). **(H&E x 250**)

Fig. (4) Photomicrograph of thyroid gland of treated OVX rat with 120 mg soybean / rat b.w. showing flattened lining epithelium of the follicles (arrow) and dilatation in most of the follicles (F). **(H&E x 250)**

Fig. (5) Photomicrograph of thyroid gland of treated OVX rat with 190 mg soybean / rat b.w. showing dilatation of the interlobular blood vessels (V), cloudy swelling and necrotic cell between the follicles (arrow). **(H&E x 250)**

4 .Discussion

The present data indicate that in comparison to the normal control group, OVX showed significant decrease of femur bone weight, femur bone Ca^{++} and serum Ca^{++}, but serum PTH significantly increased.

Compared to OVX-control group, OVX female rats fed on(60, 120, 190 mg/145 gm rat b.w) daily showed significant increase of femur bone weight, femur bone Ca^{++} and serum Ca^{++}. The increase in femur bone weight and serum Ca^{++} with all doses while femur bone Ca^{++} with 90 gm was higher than the normal control group which may lead to adverse effects. This was accompanied by a significant decrease in serum PTH of OVX female rats fed only on 90 gm soybean/70 kg human b.w.

These results agree with **Mei *et al*. (2001)** who found that soy isoflavones significantly lowered serum PTH, this decrease was associated with an increase in BMD in postmenopausal women.

A possible explanation for the increase of femur bone Ca^{++} during consumption of soybeans may be due to its ability to increase intestinal Ca^{++} absorption along with modulation of PTH levels to reach the normal control group (**Omi *et al.,* 1994)**. Also, Basal diet used in the present study consisted of vitamin D_3 which maintains mineral homeostasis and increases intestinal Ca^{++} absorption. In addition to the presence of minerals such as Ca^{++}.

Another interpretation of increased Ca^{++} content in bone may be due to soy proteins which are relatively low in the sulfur-containing amino acids and, as such, are hypothesized to be potentially to the Ca^{++} regulation and the skeleton **(Massey, 2003)**. Also, **Kaneko *et al.* (1990)** and **Messina and Messina (2000)** stated that because fewer sulfur-containing amino acids are found in soy foods, the result is a decrease in urinary Ca^{++} excretion, which was assumed to occur because of better retention of Ca^{++} in bone.

The increase of total Ca^{++} in serum may be due to the presence of protein and/or peptide in soybeans that bind to Ca^{++} and decrease urinary Ca^{++} excretion **(Arjmandi *et al.,* 1994)**. So it is logic to assume that total Ca^{++} concentration in serum was increased.

In addition, genistein aglycones showed a positive effect on bone confirmed by decrease osteoclastic resorption by numerous possible mechanisms: Induction of apoptosis **(Rassi *et al*., 2002)**, inhibition of protein tyrosine kinase **(Williams *et al.,* 1998)**, activation of protein tyrosine phosphatase **(Goa and yamaguchi, 2000)**, change in intracellular concentrations of Ca^{++} and membrane depolarization **(Okamoto *et al.,* 2001)** and increase osteoblastic formation via at least three lines of evidence: 1- Stimulation of activity, proliferation, and differentiation in cells of osteoblast lineage (**Choi *et al.,* 2001** and **Lee *et al.,* 2001)**; 2- Protection of osteoblasts from apoptosis **(Choi *et al.,* 2001)**; and 3- enhancement of bone formation rate **(Dang *et al.,* 2002)**.

Filipovic *et al*. (2010) cited that daidzein may affect bone structure indirectly through enhancement of thyroid C cell activity. Namely, calcitonin secreted from C cells is known to inhibit osteoclast activity directly through its receptors and stimulation of osteoblast activity. This hormone suppresses the number and mortility of osteoclasts **(Gao and Yamaguchi, 1999)**.

Soy isoflavones may exert similar effects as growth hormone (GH) on BMC by increasing IGF-I, which increase osteoblastic activities **(Arjmandi *et al.,* 1998** and **2003)**, the anabolic effect may result in appositional growth, resulting in an increase in bone area **(Landin-Wilhelmsen *et al.,* 2003)**.

As to the knowledge of the author no available data are present concerning femur bone weight. The increase of femur bone weight after three doses was higher than OVX- control and normal control could be explained by the presence of a fatty substance which was noticed during the practical work during grinding the bone had a creamy and greasy appearance.

The present data indicate that in comparison to OVX-control group, OVX female rats fed 120 and 190 mg soybean/145 gm rat b.w. showed significant decrease of FT_3. Serum FT_4 significantly decreased in OVX female rats fed only on 190 mg soybean /145gm rat b.w. This was accompanied by a significant increase of TSH in OVX female rats fed only on 190mg soybean /145 gm rat b.w. However, soybeans had no effect on serum total T_3 and T_4.

These results agree with studies which found decrease in serum FT_3 and increase in TSH **(Kajiya *et al.*, 2005** and **Panda *et al.*, 2009)**, decrease in serum FT_4 (**Jayagopal *et al.*, 2002**) and no significant effect in serum total T_4 and T_3 (**Mackey *et al.*, 2000; Thiagarajan *et al.*, 2000** and **Roughead *et al.*, 2005**) after consumption of soy components.

A possible explanation may be due to that soy isoflavones are goitrogenic in nature **(Divi *et al.*, 1997** and **Doerge and Sheehan, 2002)**. Indeed, **Schröder-van der Elst *et al.* (2003)** demonstrated that isoflavones decreased iodide uptake and sodium-iodide symporter (NIS) that is located in the basolateral plasma membrane of the thyrocytes and use to transport and concentrate iodide from the circulation into the thyroid follicles during early hours of treatment. The inhibition of targeted iodide uptake via NIS may reduce thyroid hormone synthesis and decrease thyroid hormone levels available for the tissues in the periphery.

Divi and Doerge (1996); Chang and Doerge (2000); Son *et al.* (2001); Doerge and Chang (2002); Doerge and Sheehan (2002); Howard (2003); Hamann *et al.* (2006) and **Hampl *et al.* (2009)** interpreted the decrease in serum T_3 and T_4 by the ability of soy isoflavones to inhibit TPO, the enzyme necessary for the iodination of tyrosine residue in the thyroglobulin molecule which undergo oxidative coupling to yield predominantly T_4 and to a minor amount of biologically active form T_3. Inhibition of TPO leads to decrease thyroid hormone synthesis. These decreased levels of thyroid hormones could then stimulate TSH secretion through the hypothalamo-pituitary-thyroid axis. The follicular cell hypertrophy induced by TSH could then lead to goiter formation **(Howard, 2003)**. In addition, **Doerge and Sheehan (2002)** found that in the presence of iodide ion, genistein covalently binds to the active side of TPO and blocks its activity by acting as alternate substrate for TPO resulting in the formation of mono-, di- and tri-iodogenistein.

Also, **Ganong (2005)** studied the impact of soy sterols in lowering the concentration of serum T_3, this could be because of the sterol which induced inhibition in peripheral conversion of T_4 to T_3, the major source of the generation of the latter hormone. This possibility was supported by a proportionate reduction in the activity of primary deiodinating enzyme, 5′DI. A significant inhibition in G-6-Pase, a marker enzyme of thyroid function **(Panda and Kar, 2006)** observed in sterol treated group, which further supported the fact that soy sterol has the potential to inhibit thyroid function, particularly the T_3 synthesis, through an inhibition in extrathyroidal conversion of T_4 to T_3. Also, the possible reason for the decrease of thyroid hormones may be due to atrophy and obliteration in thyroid follicles as seen in the present histological study Figs. (3, 4 & 5).

Bone is the major storage site for calcium in the body, and movement of calcium into and out of bone helps to determine blood calcium levels, which is critical for normal muscle and nervous system function. Calcium moves into bone as osteoblasts build new bone and out of bone as osteoclasts break down bone. When osteoblast and osteoclast activity is balanced, the movement of calcium into and out of a bone is equal.

When blood calcium levels are too low osteoclast activity increases, calcium is released by osteoclasts from bone into the blood, and blood calcium levels increase. Conversely, if blood calcium levels are too high, osteoclast activity decreases, calcium is taken from the blood by osteoclasts to produce new bone, and blood calcium levels decrease. Also, parathyroid hormone (PTH) from the parathyroid glands stimulates increased bone break down and increased blood calcium levels by indirectly stimulating osteoclast activity. PTH also increases calcium reabsorption from the urine in the kidney. PTH also stimulates the kidneys to form active vitamin D, when increases calcium absorption from the small intestine, decreasing blood calcium levels stimulates PTH secetion. In addation calcitonin, secreted from C cells of thyroid gland, decreases osteoclast activity and thus decreases blood calcium levels. Increasing blood calcium levels stimulate calcitonin secretion, these agreement with **Filipovic *et al.* (2010)** cited that daidzein may affect bone structure indirectly through enhancement of thyroid C cell activity. Namely, calcitonin secreted from C cells is known to inhibit osteoclast activity directly through its receptors and stimulation of osteoblast activity. This hormone suppresses the number and mortility of osteoclasts.

In conclusion our finding suggest that soy protein does not restore bone loss in osteopenic rats however, higher doses of iso flavones may be required to revelse the bone loss. From the all result in our study, soybean may have adverse effects on bone health and pituitary thyroid axis which is reflected by significant decrease in thyroid hormones in ovariectomized female albino rats.

Table (I): Effect of different doses of soybean on femur bone weight, femur bone Ca++, serum Ca++, E2 and PTH of ovx female albino rats treated for three months.

Parameter / *Groups*			*Femur bone weight (gm)*	*Femur bone Ca++ (mg/gm)*	*Serum Ca++ (mg/dl)*	*Total E2 (pg/ml)*	*PTH (pg/ml)*
Control groups	Normal control	Range	0.27–0.31	10.58–15.31	8.91–11.98	7.26–8.86	7–10.4
		Mean ± S.E.	0.28±0.01	12.43 ±0.41	10.24±0.28	7.94±0.48	8.71±0.59
	Sham-operation	Range	0.25–0.33	10.11–13.44	8.36–12.07	6.38 – 10.18	8.9–11
		Mean ± S.E.	0.3±0.01	11.35±0.33	10.79±0.34	8.78±1.21	9.44±0.4
		% of change	7.14 (a)	-8.69 (a)	5.37 (a)	10.58 (a)	8.38 (a)
		P value	N.S (a)	N.S (a)	N.S (a)	N.S (a)	N.S (a)
	OVX	Range	0.25 – 0.28	6.12 – 9.05	7.98–8.71	1.79 –4.99	10.3 – 12.1
		Mean ± S.E.	0.27±0.01	8±0.32	8.39±0.11	3.41±0.71	11±0.42
		% of change	- 10 (b)	-29.52 (b)	- 22.24 (b)	- 61.16 (b)	16.53 (b)
		P value	P<0.05(b)	P<0.001 (b)	P<0.001 (b)	P<0.01(b)	P<0.05(b)
Soybean treated groups	First group (60mg/145gm b.w.)	Range	0.31–0.36	8.05–12.2	9.57–12.55	2.48–5.95	7.84–11.4
		Mean ± S.E.	0.33±0.01	9.65±0.56	11.23±0.33	4.66±1.1	9.51±0.68
		% of change	22.22 (c)	20.63 (c)	33.85 (c)	36.66 (c)	-13.55 (c)
		P value	P<0.01 (c)	P<0.05 (c)	P<0.001 (c)	N.S (c)	N.S (c)
	Second group (120mg/145gm b.w.)	Range	0.31–0.36	8.63–14.88	12.54–13.44	3.96 – 6.19	5.6–11.77
		Mean ± S.E.	0.34±0.01	11.79±0.75	13.18±0.08	4.93±0.66	8.92±1.09
		% of change	25.93 (c)	47.38 (c)	57.09 (c)	44.57 (c)	-18.91 (c)
		P value	P<0.001 (c)	P<0.001 (c)	P<0.001 (c)	N.S (c)	N.S (c)
	Third group (190mg/145gm b.w.)	Range	0.32–0.35	10.4–18.38	12.07–14.81	4.21–5.84	6.6–10.2
		Mean ± S.E.	0.34±0.01	14.88±0.82	13.36±0.26	4.96±0.48	8.53±0.62
		% of change	25.93 (c)	86 (c)	59.24 (c)	45.45 (c)	-22.45 (c)
		P value	P<0.001 (c)	P<0.001 (c)	P<0.001 (c)	N.S (c)	P<0.05 (c)
		ANOVA	F = 10.76 P < 0.001	F = 17.34 P < 0.001	F = 39.14 P < 0.001	F = 6.79 P < 0.01	F = 1.5 N.S

(a)compared with normal control group (b) compared with sham-operated groups

(c) compared with OVX group

N.S: non-significant P: probability S.E.: standard error

Table (II): Effect of different doses of soybean on on serum TSH and thyroid hormones of ovariectomized female albino rats treated for three months.

Parameter / *Groups*			*TSH µIU/ml)*	*Free T_4 ng/dl*	*Free T_3 pg/ml*
Control groups	Normal control	Range	0.1 – 0.2	1.41 – 1.65	4.72 – 5.4
		Mean ± S.E.	0.15 ± 0.01	1.56 ± 0.08	5.01 ± 0.14
	Sham-operation	Range	0.15 – 0.19	1.5 – 1.74	4.42 – 4.8
		Mean ± S.E.	0.17 ± 0.01	1.65 ± 0.05	4.57 ± 0.12
		% of change	13.33 (a)	5.77 (a)	- 8.78 (a)
		P value	N.S (a)	N.S (a)	N.S (a)
	OVX	Range	0.15 – 0.31	1.49 – 1.6	3.83 – 4.86
		Mean ± S.E.	0.18 ± 0.03	1.53 ± 0.04	4.46 ± 0.32
		% of change	5.88 (b)	- 7.27 (b)	-2.41 (b)
		P value	N.S (b)	N.S (b)	N.S (b)
Soybean treated groups	First group (60mg/145gm b.w.)	Range	0.15 – 0.31	1.38 – 1.76	4.2 – 4.7
		Mean ± S.E.	0.18 ± 0.02	1.5 ± 0.11	4.45 ± 0.14
		% of change	0 (c)	-1.96 (c)	-0.22 (c)
		P value	N.S (c)	N.S (c)	N.S (c)
	Second group (120mg/145gm b.w.)	Range	0.15 – 0.33	1.4 – 1.52	3.72 – 4
		Mean ± S.E.	0.21 ± 0.03	1.47 ± 0.04	3.86 ± 0.08
		% of change	16.67 (c)	-3.92 (c)	-13.45 (c)
		P value	N.S (c)	N.S (c)	N.S (c)
	Third group (190mg/145gm b.w.)	Range	0.19 – 0.36	1.35 – 1.43	3.52 – 3.6
		Mean ± S.E.	0.27 ± 0.02	1.38 ± 0.03	3.56 ± 0.02
		% of change	50 (c)	-9.8 (c)	-20.18 (c)
		P value	P < 0.05 (c)	P < 0.05 (c)	P < 0.05 (c)
		ANOVA	F = 4.71 P < 0.01	F = 2.28 N.S	F = 10.36 P < 0.001

(a)compared with normal control group (b) compared with sham-operation

(c) compared with OVX group

N.S: non-significant P: probability S.E.: standard error

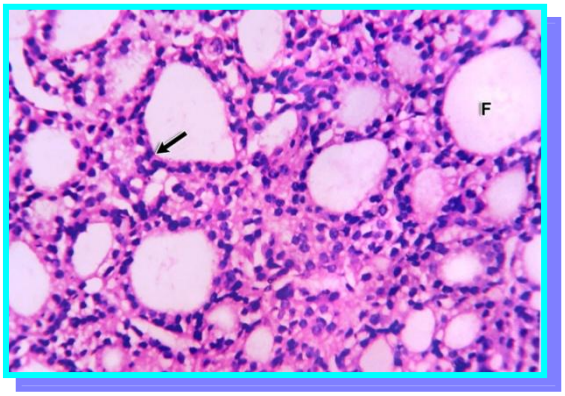

Fig. (1) Photomicrograph of thyroid gland of normal control rat showing normal histological structure of the active follicles (F) with cubodial lining epithelium (arrow). (H&E x 250)

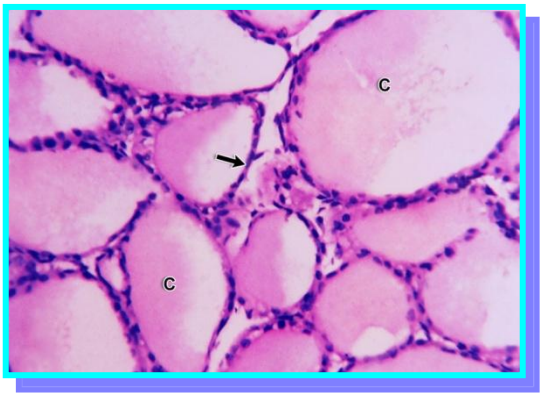

Fig. (2)Photomicrograph of thyroid gland of OVX-control rat showing inactive cystic dilatation with massive amount of colloid in the follicular lumen (C) and flattened lining epithelium (arrow). (H&E x 250)

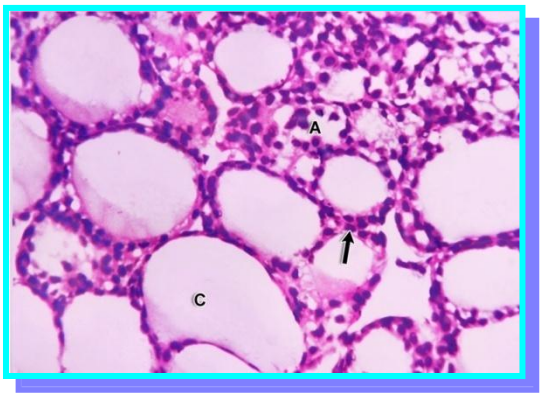

Fig. (3) Photomicrograph of thyroid gland of treated OVX rat with 60mg soybean / 145 gm /rat b.w. showing cubodial lining epithelium of the follicles (arrow), atrophy and obliteration in some follicles (A) while other containing eosinophilic colloid (C).

(H&E x 250)

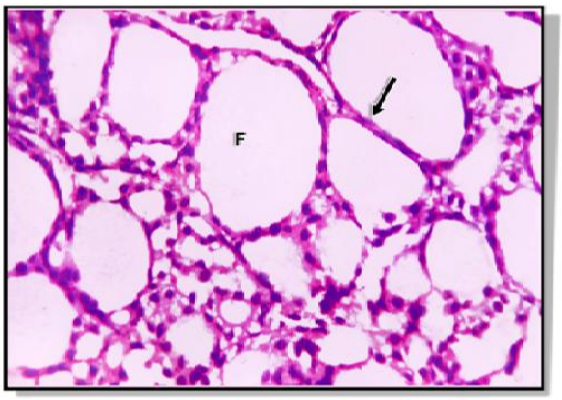

Fig. (4) Photomicrograph of thyroid gland of treated OVX rat with 120mg soybean / 145gm/rat b.w. showing flattened lining epithelium of the follicles (arrow) and dilatation in most of the follicles (F). (H&E x 250)

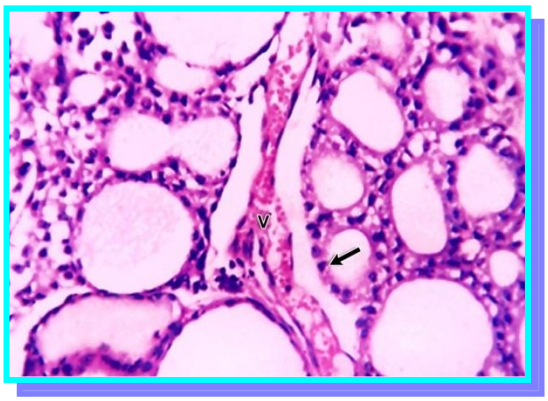

Fig. (5) Photomicrograph of thyroid gland of treated OVX rat with 190mg soybean / 145gm/ rat b.w. showing dilatation of the interlobular blood vessels (V), cloudy swelling and necrotic cell between the follicles (arrow). (H&E x 250)

References

Ahlborg, H.G.; Johnell, O. and karlsson, M.K. (2004): "Long term effects of oestrogen therapy on bone loss in postmenopausal women: a 23 year prospective study". BJOG. 111: 335-339.

Alekel, D.L.; Germain, A.S.; Peterson, C.T.; Hanson, K.B.; Stewart, J.W. and Toda, T. (2000): "Isoflavone-rich soy protein isolated attenuates bone loss in the lumbar spine of perimenopausal women". Am. J. Clin. Nutr. 72: 844-852.

Arjmandi, B.H.; Getlinger, M.J.; Goyal, N.V.; Alekel, L.; Hasler, C.M.; Juma, S.; *et al.* (1998): "The role of soy protein with normal or reduced isoflavone content in reserving bone loss induced by ovarian hormones deficiency in rats". Am. J. Clin. Nutr. 68: 1358S-1363S.

Arjmandi, B.H.; Hollis, B.W. and Kalu, D.N. (1994): "*In vivo* effect of 17β-estrdiol on intestinal calcium absorption in rats". Bone Miner. 26: 181-189.

Arjmandi, B.H.; Khalil, D.A.; Smith, B.J.; Lucas, E.A.; Juna, S.; Payton, M.E. and Wild, R.A. (2003): "Soy protein has a greater effect on bone in postmenopausal women not on hormone replacement therapy, as evidenced by reducing bone resorption and urinary calcium excretion". J. Clin. Endocinol. Meta. 88: 1048-1054.

Atmaca, A.; Kleerekoper, M.; Bayraktar, M. and Kucuk, O. (2008): "Soy isoflavones in the management of postmenopausal osteoporosis". Menopause 15: 748-757.

Azza,M.E. , Hassan ,A., NERMIN ,S. Gharib ,S. (2014) .

Osteoprotective effect of soybean and seesame oil in overiectomized rats via estrogen –like mechanism cytoechnology ,66:335-343.

Bahr, J.M.; Nakai, M.; Rivera, A.; Walsh, J.; Evans, G.L.; Lotinun, S.; Turner, R.T.; Black, M. and Jeffery, E.H. (2005): "Dietary soyprotein and isoflavones : minimal beneficial effects on bone and no effect on the reproductive tract of sexually mature ovariectomized Sprague-Dawly rats. Menopause 12: 165-173.

Baloch, Z.; Carayon, P.; Conte-Devolx, B.; Demers, L.M.; Feldt-Rasmussen, U.; Henry, J.F.; *et al.* (2003): "Guidelines Committee, National Academy of Clinical Biochemistry (NACB). Laboratory medicine practice guide lines (LMPG). Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid 13: 3-126.

Barlow, D.H. (2007): "Osteoporosis guidelines". Climacteric 10: 79-82.

Beck, V.; Unterrieder, E.; Erenn, L.; Kubelka, W. and Jungbauer, A. (2003): "Comparsion of hormonal activity (estrogen, androgen and progestin) of standard plant extracts for large scale use in hormone replacement therapy". J. Steroid Biochem. Mol. Biol. 84: 259 – 268.

Bitto, A.; Polito, F.; Atteritano, M.; Altavilla, D.; Mazzaferro, S.; Marini, H.; Adamo, E.B.; D'Anna, R.; Granese, R.; Corrado, F.; Russo, S.; Minutoli, L. and Squadrito, F. (2010): "Genistein aglycone does not affect thyroid function: results from a three-year, randomized, double-blind, placebo-controlled trial. J. Clin. Endocrinol. Metab. 95: 3067-3072.

Breitman, P.L.; Fonseca, D.; Cheung, A.M.; *et al*. (2003): "Isoflavones with supplemental calcium provide greater protection against the loss of bone mass and strength after ovariectomy compared to isoflavones alone". Bone 33: 597- 605.

Brink, E.; Coxam, V.; Robins, S.; Wahala, K.; Cassidy, A. and Branca, F. (2008): "Long-term consumption of isoflavone-enriched food does not affect bone mineral density, bone metabolism, or hormonal status in early postmenopausal women. Am. J. Clin. Nutr. 87: 761-770

Cassidy, A.; Albertazzi, P.; Nielsen, I.L.; Hall, W.; Williamson, G.; Tetens, I.; Atkins, S.; Cross, H.; Manios, Y.; Wolk, A.; Steiner, C. and Branca, F. (2006): "Critical review of health effects of soyabean phyto-oestrogens in post-menopausal women". Proc. Nutr. Soc. 65:76–92.

Chan, M.C.; Anderson, M. and Lau, E.M.C. (2003): "Exercise interventions: defusing the world's osteoporosis time bomb". Bull World Health Organ. 81: 827-830.

Chang, H.C. and Doerge, D.R. (2000): "Dietary genistein inactivates rat thyroid peroxidase *in vivo* without an apparent hypothyroid effect". Toxicol. Appl. Pharmacol. 168: 244–252.

Charoenphandhu, N.; Tudpor, K.; Thongchote, K.; Saengamnart, W.; Puntheeranurak, S. and Krishnamra, N. (2007): "High-calcium diet modulates effect of long-term prolactin exposure on the cortical bone calcium content in ovariectomized rats. Am. J. Physiol. Endocrinol. Metab. 292: E443-E452.

Cheong, J.M.K.; Martin, B.R.; Jackson, G.S.; Elmore, D.; McCabe, G.P.; Nolan, J.R.; Barnes, S.; Peacock, M. and Weaver, C.M. (2006): "Soy isoflavones do not affect bone resorption in postmenopausal women". J. Clin. Endocrinol. Metab. 92: 577-582.

Clifton-Bligh, P.B.; Baber, R.J.; Fulcher, G.R.; Nery, M.L. and Moreton, T. (2001): "The effect of isoflavones extracted from red clover (Rimostil) on lipid and bone metabolism. Menopase 8: 259-265.

Dang, Z.C.; Van Bezooijen, R.L.; Karperien, M.; Papapoulos, S.E. and Lowik, C.W. (2002): "Exposure of KS483 cells to estrogen enhances osteogenesis and inhibits adipogenesis". J. Bone Miner. Res. 17: 394-405.

Divi, R.L. and Doerge, D.R. (1996): "Inhibition of thyroid peroxidase by dietary flavonoids". Chem. Res. Toxicol. 9: 16-23.

Divi, R.L.; Chang, H.C. and Doerge, D.R. (1997): "Antithyroid isoflavones from soybean. Biochem. Pharmacol. 54: 1087–1096.

Doerge, D.R. and Chang, H.C. (2002): "Inactivation of thyroid peroxidase by soy isoflavones, *in vitro* and *in vivo*. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 777: 269-279.

Doerge, D.R. and Sheehan, D.M. (2002): "Goitrogenic and estrogenic activity of soy isoflavones". Environ. Health. Perspect. 110: 349-353.

Figard, H.; Mougin, F.; Gaume, V. and Berthelot, A. (2006): "Combined intervention of dietary soybean proteins and swing training: effects on bone metabolism in ovariectomized rats. J. Bone Miner. Metab. 24: 206-212.

Filipovic, B.; Sosic-Jurjevic, B.; Ajdzanovic, V.; Brkic, D.; Manojlovic-Stojanoski, M.; Milosevic, V. and Sekulic, M. (2010): "Daidzein administration positively affects thyroid C cells and bone structure in orchidectomized middle-aged rats". Osteoporos. Int. 21: 1609–1616**.**

Fonseca, D. and Ward, W.E. (2004): "Daidzein together with high calcium preserve bone mass and biochemical strength at multiple sites in ovariectomized mice". Bone 35: 489-497.

Ganong, W.E. (2005): Editor, Review of medical physiology (22nd ed.), Appleton and Lange, Connecticut: 303-309.

Gao, Y.H. and Yamaguchi, M. (2000): "Suppressive effect of genistein on rat bone osteoclasts: involvement of protein kinase inhibition and protein tyrosine phosphatase activation. Int. J. Mol. Med. 5: 261–267.

Gindler, M. (1972): "Colorimetric determination of serum calcium. Am. J. Clin. Path. 58: 376-382.

Hamann, I.; Seidlova-Wuttke, D.; Wuttke, W. and Köhre, J. (2006): "Effects of isoflavonoid and other plant-derived compounds on the hypothalamus-pituitary-thyroid hormone axis. Maturitas 55: S14-S25.

Hampl, R.; Ostatnikova, D.; Celec, P.; Putz, Z.; Bílek, R.; Lapcík, O. and Matucha, P. (2009): "Chapter 38 -correlation between soy phytoestrogens and thyroid laboratory parameters: implications for iodine nutrition. Comprehensive Handbook of Iodine Nutr. Biochem. Pathol. Therap. Asp.: 353-363.

Hays, J.; Ockene, J.K.; Brunner, R.l.; Kotchen, J.M.; Mansan, J.E.; Patterson, R.E.; Aragaki, A.K.; Shumarker, S.A.; Brzyski, R.G.; Lacroix, A.Z.; Granek, I.A. and Valanis, B.G. (2003): "Effects of Estrogen plus progestin on Health-Related Quality of life". N. Engl. J. Med. 348: 348 – 364.

Heaney, R.P. (2002): "Effects of caffeine on bone and the calcium economy". Food Chem. Toxicol. 40: 1263-1270.

Hohenhaus, M.H.; Mc Garry, K.A. and Col, N.F. (2007): "Hormone therapy for the prevention of bone loss in menopausal women with osteopenia: is it a viable option?. Drugs 67: 2311-2321.

Howard, B. (2003): "Thyroid Hormones. Biochemistry Lecture Handout. David Geffen School of Medicine at UCLA, Los Angeles, CA.

J. of Food Sci 71, Nr. 1: C95-C101.

Jayagopal, V.; Albertazzi, P.; Kilpatrick, E.S.; Howarth, E.M.; Jennings, P.E.; Hepburn, D.A. and Atkin, S.L. (2002): "Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes". Diabet. Care. 25: 1709-1714.

Jayo, M.J.; Anthony, M.S.; Register, T.C.; *et al.* (1996): "Dietary soy isoflavones and bone loss: a study in ovariectomized monkeys". J. Bone Miner. Res. 11: S228 (abstr.).

Kajiya, H.; Takekoshi, S.; Miyai, S.; Ikeda, T.; Kimura, S. and Osamura, R.Y. (2005): "Dietary Soybean Enhances Pit-1 Dependent Pituitary Hormones Production in Iodine Deficient Rats". J. Mol. Histol. 36: 265-274.

Kaneko, K.; Masaki, U.; Aikyo, M.; Yabuki, K.; Hega, A.; Matoba, C.; *et al.* (1990): "Urinary calcium and calcium balance in young women affected by high protein diet of soy protein isolate and adding sulfur-containing amino acids and/or potassium". J. Nutr. Sci. Vitaminol. 36: 105–116.

Kao, P.C.; Grant, C.S.; Klee, G.G. and Khosla, S. (1992): "Clinical performance of parathyroid hormone immunometric assays". Mayo. Clin. Proc. 67: 637-645.

Katzung, B.G. (2001): "Endocrine Drug. Eighth edition. Basic & Clinical pharmacology. New York : Mc Graw – Hill: 625 – 735.

Kenny, A.M.; Mangano, K.M.; Abourizk, R.H.; Bruno, R.S.; Anamani, D.E.; Kleppinger, A.; Walsh, S.J.; Prestwood, K.M. and Kerstetter, J.E (2009): "Soy proteins and isoflavones affect bone mineral density in older women: a randomized controlled trial". Am. J. Clin. Nutr. 90: 234- 242.

Kim, M.S. and Lee, Y.S. (2005): "Effects of soy isoflavone and/or estrogen treatments on bone metabolism in ovariectomized rats". J. Med. Food 8: 439-445.

Landin-Wilhelmsen, K.; Nilsson, A.; Bosaeus, I. and Bengtsson, B.A. (2003): "Growth hormone increases bone mineral content in postmenopausal osteoporosis: a randomized placebo-controlled trial. J. Bone Miner Res. 18: 393-405.

Lee, S.J.; Ahn, J.K.; Kim, S.H.; Kim, J.T.; Han, S.J.; Jung, M.Y. and Chung, I.M. (2003) : "Variation in isoflavones of soybean cultivars with location and storage duration". J. Agric. Food Chem. 51: 3382 – 3389.

Lee, Y.S.; Chen, X. and Anderson, J.J.B. (2001): "Physiological concentrations of genistein stimulate the proliferation and protect against free radical-induced oxidative damage of MC3T3-E1 osteoblast-like cells". Nutr. Res. 21: 1287-1298.

Levine, J.P. (2007): "Effective strategies to identify postmenopausal women at risk for osteoporosis". Geriatr. 62: 22-30.

Liewendhal K. (1990): "Assessment of thyroid status by laboratory methods: development and prepectives". Scand. J. Clin. Lab. Invest. Suppl. 201: 83-92.

Lin, C.C Pey,S. Wu., David,WM.L ; , Chang,CK and Yi-Shyan Chen,YS. (2012) Quality, antioxidative ability, and cell proliferation –enhancing activity of fermented black soybean broths with various supplemental culture medium.

Ma, D.F.; Quine, L.Q.; **Wang, P.Y. and Katoh, R. (2008):** "Soy isoflavones intake inhibits bone resorption and stimulates bone formation in menopausal women: meta-analysis of randomized controlled trials". Eur. J. Clin. Nutr. 62: 155-161.

Mackey, R.; Ekangaki, A. and Eden, J.A. (2000): "The effects of soy protein in women and men with elevated plasma lipids". Biofactors. 12: 251-257.

Marie, P. (2001): "Différenciation, fonction et contrôle de l`ostéoblaste". M.S. Med. Sci. 17: 1252-1259.

Marini, H.; Bitto, A.; Altavilla, D.; Burnett, B.P.; Polito, F.; Di Stefano, V.; Minutoli, L.; Atteritano, M.; Levy, R.M.; Frisina, N.; Mazzaferro, S.; Frisina, A.; D'Anna, R.; Cancellieri, F.; Cannata, M.L.; Corrado, F.; Lubrano, C.; Marini, R.; Adamo, E.B. and Squadrito, F. (2010): "Efficacy of genistein aglycone on some cardiovascular risk factors and homocysteine levels: A follow-up study". Nutr. Metab. Cardiovasc. Dis. 20: 332-340.

Marini, H.; Minutoli, L.; Polito, F.; Bitto, A.; Altavilla, D.; Atteritano, M.; *et al.* (2008): "OPG and sRANKL serum concentrations in osteopenic postmenopusal women after 2-years genistein administration". J. Bone Miner. Res. 23: 715-720.

Massey, L.K. (2003): "Dietary animal and plant protein and human bone health: a whole foods approach". J. Nutr. 133: 862S–865S.

McCue, P. and Kalidas, S. (2004): "Health benefits of soy isoflavonoids and strategies for enhancements: a review". Critical Reviews in food Sci. Nutr. 44: 361 – 367.

McKane, W.R.; Khosla, S.; Risteli, J.; Robins, S.P.; Muhs, J.M. and Riggs, B.L. (1997): "Role of estrogen deficiency in pathogensis of secondary hyperparathyroidism and increased bone resorption in elderly women". Proc. Assoc. Am. Physicians 109: 174-180.

Mei, J.; Yeung, S.S. and Kung, A.W. (2001): "High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women". J. Clin. Endocrinol. Metab. 86: 5217–5221

Messina, M. (2002): "Soy foods and soybean isoflavones and menopausal health. Nutr. Clin. Care. 5: 272 – 282.

Messina, M. and Messina, V. (2000): "Soyfoods, soybean isoflavones, and bone health: a brief overview. J. Ren. Nutr. 102: 63–68.

Nakai, M.; Cook, L.; Pyter, L.M.; Black, M.; Sibona, J.; Turner, R.T.; Jeffery, E.H. and Bahr, J.M. (2005): "Dietary soy protein and isoflavones have no significant effect on bone and a potentially negative effect on the uterus of sexually mature intact Sprague-Dawley female rats. Menopause 12: 291-298.

Nelson, H.; Haney, E.; Humphrey, L.; *et al.* (2005): "Management of menopause – related symptoms. Evidence Report /Technology Assessment, No. 120. Rockville, MD : Agency for Healthcare Research and Quality.

Nussey, S.S. and Whitehead, S.A. (2001): "Endocrinology: an intergrated approach. BIOS Scientific Pulishers Limited, Oxford, UK. P. 376.

Okamoto, F.; Okabe, K. and Kajiya, H. (2001): "Genistein, a soybean isoflavone, inhibits inward rectifier K(+) channels in rat osteoclasts. Jpn. J. Physiol. 51: 501–509.

Omi, N.; Aoi, S.; Murata, K. and Eza, W.I. (1994): "Evaluation of the effect of soybean milk and soybean peptide on bone metabolism in the rat model with ovariectomized osteoporosis. J. Nutr. Sci. Vitamino. (Tokyo) 40: 201–211.

Panda, S. and Kar, A. (2006): "Evaluation of the anti-thyroid, antioxidative and antihyperglycaemic activity of scopoletin from Aegle maremelos leaves in hyperthyroid rats. Phytother. Res. 20: 1103-1105.

Panda, S.; Kar, A. and Patil, S. (2009): "Soy sterols in the regulation of thyroid functions, glucose homeostasis and hepatic lipid peroxidation in mice". Food Res. Int. J. 42: 1087-1092.

Park,M.H.;Min,K,J.;Kim,M. and Lee,J.H.(2012): Modification of isoflavone profiles in a fermented soy food with almond powder. Journal of food science 71,Nr.1: C128-C134.

Perez, A.V.; Picotto, G.; Carpentieri, A.R.; Rivoira, M.A.; Peralta Lopez, M.E. and Tolosa de Talamoni, N.G. (2008): "Minireview on regulation of intestinal calcium absorption: emphasis on molecular mechanisms of transcellular pathway". Digestion 77: 22–34.

Peverill, R.E. (2003): "Hormone therapy and venous thromboembolism". Best. Proct. Res. Clin. Endocrinol. Metab. 17: 149 – 164.

Picherit, C.; Bennetallpelissero, C.; Chanteranne, B.; Labecque, P.; Davicco, M.J.; Barlet, J.P.; *et al*. (2001): "Soybean isoflavones dose-dependently reduce bone turnover but do not reverse established osteopenia in adult ovariectomized rats". J. Nutr. 131: 723-728.

Picherit, C.; Horcajada, M.N.; Mathey, J.; *et al*. (2003): "Isoflavones consumption does not increase the bone mass in osteopenic obese female zucker rats". Ann. Nutr. Metab. 47: 70-77.

Puntheeranurak, S.; Charoenphandhu, N. and Krishnamra, N. (2006): "Enhanced trabecular-bone calcium deposition in female rats with a high physiological dose of prolactin diminishes after ovariectomy". Can. J. Physiol. Pharmacol. 84: 993-1002.

Rassi, C.M.; Lieberherr, M.; Chaumaz, G.; *et al.* (2002): "Down-regulation of osteoclast differentiation by daidzein via caspase 3". J. Bone Miner. Res. 17: 630–638.

Reeves, P.G.; Nielson, F. and Fahey, G.C. (1993): "Purified Diets for Laboratory Rodents : Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent diet". J. Nutr. 123: 1939-1951.

Riggs, B.L.; Khosla, S. and Melton, L.J. (2002): "Sex steroids and the construction and conservation of the adult skeleton". Endocrinol. Rev. 23: 279–302.

Roughead, Z.K.; Hunt, J.R.; Johnson, L.K.; Badger, T.M. and Lykken, G.I. (2005): "Controlled substitution of soy protein for meat protein: effects on calcium retention, bone, and cardiovascular health indices in postmenopausal women". J. Clin. Endocrinol. Metab. 90: 181-189.

Sandra, M. S., Marie ,N. H. and Elizabeth, O.(2013)

Phytonutrients for bone health during ageing

Br J Clin Pharmacol. Mar; 75(3): 697–707.

Schwartz, M.W. (2001): "Brain pathways controlling food intake and body weight". Exp. Biol. Med. (Maywood) 226: 978-981.

Setchell, K.D.R. and Lydeking-Olsen, E. (2003): "Dietary phytoestrogens and their effect on bone: evidence from *in vitro* and *in vivo*, human observational and dietary intervention studies". Am. J. Clin. Nutr. 78: 593-609.

Shi, L.N. and Su, Y.X. (2000): "The effect of soybean isoflavones on bone loss in ovariectomized rat". Acta. Nutrimenta. Sinica. 22: 113-118.

Silverberg, S.J. and Bilezikian, J.P. (1994): "Parathyroid function and responsiveness in osteoporosis". In: Bilezikian, J.P.; Marcus, R.; Levine, M.A. (eds.), the parathyroid Basic and Clinical Concepts. New York: Raven Press: 805-812.

Son, H.Y.; Nishikawa, A.; Ikeda, T.; Imazawa, T.; Kimura, S. and Hirose, M. (2001): "Lack of effect of soy isoflavone on thyroid hyperplasia in rats receiving an iodine-deficient diet". Jpn. J. Cancer Res. 92: 103-108.

Strewler G.J. (2001): "Local and systemic control of the osteoblast". J. Clin. Invest. 107: 271-272.

Taku, K.; Melby, M.K.; Takebayash, J.; Mizuno, S.; Ishimi, Y.; Omori, T. and Watanabe, S. (2010): "Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: Meta-analysis of randomized controlled trials". J. Clin. Nutr. 19: 33-42.

Thiagarajan, D.G.; Bennink, M.R. and Bourquin, L.D. (2000): "No adverse changes in blood chemistry parameters in humans after consuming soy protein for one year". FASEB. J. Abstract 160:2.

Wangen, K.E.; Duncan, A.M.; Xu, X. and Kurzer, M.S. (2001): **"Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women". J. Clin. Nutr. 73: 225-231.**

Williams, J.P.; Jordan, S.E.; Barnes, S.; *et al.* (1998): "Tyrosine kinase inhibitor effects on avian osteoclastic acid transport". Am. J. Clin. Nutr. 68: 1369S–1374S.

Xing, S.; Cekan, S.Z.; Diczfalusy, U.; et al. (1983): "Validation of radioimmunoassay for estradiol-17β by isotope dilution-mass spectrometry and by a test of radiochemical purity". Clin. Chim. Acta. 135: 189-201.

Ye, Y.B.; Tang, X.Y.; Verbruggen, M.A. and Su, Y.X. (2006): "Soy isoflavones attenuate bone loss in early postmenopausal Chinese women: a single-blind randomized, placebo-controlled trial. Eur. J. Nutr. 45: 327-334.

Young, D.S.; Pestaner, L.C. and Gilberman, U. (1975): "Young effects of drugs on clinical laboratory tests". Clin. Chem. 21: 3660.

Zhang, Y., T. Ojima and C. Murata (2007). Calcium intake pattern among Japanese Women across five stages of health behavior change. J. Epidemiol., 17: 45-53.

Zhang, Y.; Yin, L. and Hillgartner, F.B. (2001): "Thyroid hormone stimulates acetyl-coA carboxylase-alpha transcription in hepatocytes by modulating the composition of nuclear receptor complexes bound to a thyroid hormone response element". J. Biol. Chem. 276: 974-983.